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AMPHIPHILE DIFFUSION IN MODEL MEMBRANE SYSTEMS STUDIED BY PULSED NMR

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The translational diffusion of the amphiphilic molecules in a number of lyotropic liquid crystalline phases has been measured with the pulsed NMR pulsed magnetic field gradient method. The amphiphiles studied were soaps, monoglycerids and lecithins. Measurements were performed both for oriented lamellar and for cubic phases. The order of magnitude of the diffusion coefficients was found to be the same as in neat liquids of analogous compounds. It was also found that the difussion coefficient depend markedly on the amphiphile end group in a way that parallels the area per polar head group as determined in X-ray studies. When corrections for geometrical factors has been made the diffusion rate is approximately equal in cubic and lamellar phases containing the same amphiphile.

1. Introduction

Much interest has been focussed on lyotropic liquid crystals as model systems for the understanding of the physical properties of biological membranes. X-ray and calorimetric techniques have been used to study the structure and the stability regions for different phases, while spectroscopic methods have provided detailed information on the molecular level. However, there exists few reliable determinations of amphiphile translational diffusion in these systems, mainly due to experimental difficulties. In micellar solutions the diffusion coefficient has been determined by the radioactive tracer technique [1], in phases of cubic symmetry by some NMR field gradient technique [2–4] and for lamellar systems indirectly using the ESR spin-label method [5, 6] or by phosphorescence quenching [7].

In the lamellar systems, which are the most interesting ones from the model membrane point of view, the standard NMR techniques cannot be used in a straightforward way to measure the translational diffusion. Due to the presence of static dipolar couplings the effective T_2 is too short for such measurements to be feasable. This problem can be circumvented by using samples oriented at the magic angle (54.7°) with respect to the magnetic field [8,9] so that the static dipolar couplings vanish. With such an oriented sample conventional pulsed gradient pulsed NMR techniques [10] can be used to determine the diffusion coefficient.

In this paper we present measurements of the translational diffusion of the amphiphilic molecules in a number of lamellar and cubic lyotropic liquid crystalline systems. In this way effects of amphiphile polar head groups, carbon chain length and phase structure on the diffusion rate is studied.

2. Experimental

The preparation of the samples and the macroscopical orientation of the lamellar systems were performed as described previously [11,12]. It is of great importance to eliminate as much as possible of free ^1H and compounds containing exchangeable hydrogens were treated with excess D_2O several times before use. For a successful measurement a good orientation of the sample is also needed. Fig. 1 shows the high resolution ^1H NMR spectrum for some of the oriented systems at the magic angle and at an arbitrary orientation. It is seen that a comparatively narrow signal can be obtained but also that the water peak is small.

The diffusion coefficients were measured with the pulsed magnetic field gradient method described by Stejskal and Tanner [10] at 61 MHz using a Bruker 322 s pulsed NMR spectrometer equipped with a

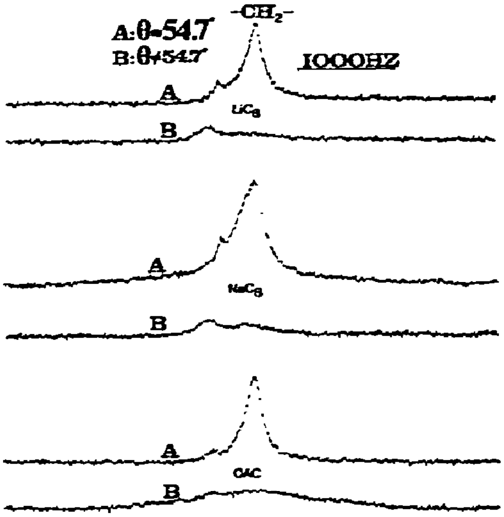

Fig. 1. High resolution ^{1}H NMR spectra of oriented samples of lithium and sodium octanoate and of octyl ammonium hydrochloride.

Bruker pulsed magnetic field gradient unit. A schematic illustration of the experiment is presented in fig. 2. The echo amplitude E observed at time 2τ is, in the presence of a magnetic field gradient, g, attenuated according to the equation [10]

$$\ln E_g/E_0 = -(\gamma g\delta)^2 D(\Delta - \delta/3)\,, \qquad (1)$$

where E_g/E_0 is the echo attenuation. The definitions of Δ and δ are shown in fig. 2 and γ is the magnetogyric ratio. The diffusion coefficients, D, were obtained by varying the gradient pulse length δ between 1.2 and 9 ms while keeping the magnetic field gradient g and the time between the gradient pulses Δ constant at approximately 2 T m^{-1} and 18 ms, respectively. The values of δ and Δ were measured visually on an oscilloscope. The lower limit of δ was chosen so as not to distort the rectangular shape of the gradient pulse. One to three echoes were accumulated in at least three separate determinations on a signal averaging unit. The effective diffusion coefficient, D, was calculated from a least square determination of the slope in a plot of $\ln E_g$ as a function of δ^2 $(\Delta - \delta/3)$ for both the sample and a glycerol reference. The ratio between the slope and the known diffusion coefficient of glycerol [13] then determined the diffusion coefficient for the sample.

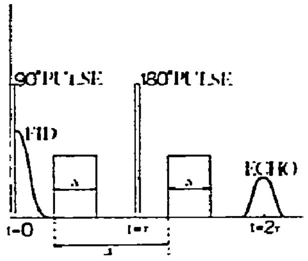

Fig. 2. Schematic representation of the pulse sequence. δ is the length of and Δ the spacing between the field gradient pulses.

3. Results

The effective amphiphile translational diffusion coefficients measured at 24°C are summarized in table 1. Some recently reported [4,9] related data are also included for comparison. The random error in D is estimated to ±20%. There may also be a systematic error mainly due to the uncertainty in the diffusion coefficient of the reference.

4. Discussion

The NMR experiment measures the diffusion in the direction of the magnetic field gradient. For anisotropic systems the diffusion coefficient is orientation dependent and in a lamellar system, for example, two constants D_L and $D_\perp$ describe the diffusion. D_L is the lateral diffusion coefficient for motion parallel with the lamellae and $D_\perp$ determine the diffusion perpendicular to the lamellae. For such a system (c.f. fig. 3)

$$D = D_L \sin^2\theta + D_\perp \cos^2\theta \,. \qquad (2)$$

For a lamellae oriented at the magic angle $\sin^2\theta = 2/3$. In amphiphilic lamellar systems $D_\perp$ should be orders of magnitudes smaller than D_L and the second term in eq. (2) can be neglected. The lateral diffusion coefficients calculated from eq. (2) are presented in the second column of table 1.

For cubic phases with continuous amphiphile regions without diffusion constraints

$$D = 1/3\, \overline{\mathrm{Tr}\, \tilde{D}}\,. \qquad (3)$$

Table 1
Measured amphiphile translational diffusion coefficient in different lyotropic liquid crystals. All measurements were made relative to the diffusion of glycerol [13]. Temperature 24°C

Sample composition (% w/w)		Phase	D (m^2/s)	$D_{\perp}$ (m^2/s)	$D_{\parallel}$ (m^2/s)
AOT [a)] D_2O	75.0 25.0	cubic	3.4×10^{-12}		1.0×10^{-11}
AOT [a)] D_2O	60.0 40.0	lamellar	1.8×10^{-11}	2.7×10^{-11}	
C_8NH_3Cl D_2O	72.0 28.0	lamellar	2.2×10^{-10}	3.3×10^{-10}	
C_7COOK D_2O	60.0 40.0	cubic	8.8×10^{-11}		2.6×10^{-10}
C_7COONa octane D_2O	39.4 4.3 56.3	cubic	1.3×10^{-12}		(3.9×10^{-12})
C_{18}-monoglyceride D_2O	77.9 22.1	cubic	5.9×10^{-12}		1.8×10^{-11}
C_{18}-monoglyceride D_2O	92.9 7.1	lamellar	5.1×10^{-12}	7.4×10^{-12}	
C_8-monoglyceride D_2O	90.0 10.0	lamellar	1.9×10^{-11}	2.9×10^{-11}	
C_7COOLi $C_{10}OD$ D_2O	20.0 40.0 40.0	lamellar	1.3×10^{-10}	2.0×10^{-10}	
C_7COONa $C_{10}OD$ D_2O	22.0 40.0 38.0	lamellar	1.4×10^{-11}	2.1×10^{-10}	
C_8SO_4Na $C_{10}OD$ D_2O	30.9 35.8 33.3	lamellar	2.3×10^{-11}	3.5×10^{-11}	
EYL [b,c)] Na-cholate D_2O	50.0 26.0 24.0	cubic	7.0×10^{-13}		2.1×10^{-12}
EYL Cholesterol D_2O	61.0 19.0 20.0	lamellar	4.8×10^{-12}	7.1×10^{-12}	
DLL [c,d,e)] Na-cholate D_2O	50.0 26.0 24.0	cubic	2.4×10^{-12}		7.2×10^{-12}
DLL [e,f)] D_2O	75.0 25.0	lamellar	8.2×10^{-12}	1.2×10^{-11}	

[a)] AOT = Aerosol OT. [b)] EYL = egg yolk lecithin. [c)] From ref. [4]. [d)] DLL = dilauroyllecithin.
[e)] At 50°C. [f)] From ref. [9].

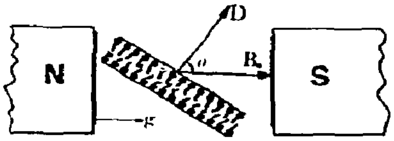

Fig. 3. Schematic illustration of the orientation of the lamellar relative to the magnetic field, B_0, and the magnetic field gradient, g.

Here $\tilde{D}$ is the local diffusion tensor, and the bar denotes an average over all the sites in a unit cell of the liquid crystal. For cubic phases built of tetrahedrally arranged rods [14] only the diffusion along the rod, $D_{\|}$, will contribute to the transport of spins and in this case [4]

$$D = D_{\|}/3 \,. \tag{4}$$

For cubic phases where amphiphile diffusion in two directions within the aggregates contribute to the transport of spins in the magnetic field gradient

$$D = 2D_{\|}/3 \,. \tag{5}$$

In table 1 the values for $D_{\|}$ in column three are calculated using eq. (4).

One difficulty with the interpretation of some of the diffusion coefficients in table 1 is that two types of amphiphilic molecules are present. This is unfortunately usually necessary for a lamellar phase to form. The echo observed at 2τ has in principle contributions from all protons in the sample. In practice, however, it is the compound with sharpest peak in the ^1H NMR spectrum and with the slowest diffusion that contributes most to the measured D. For systems where cholate or cholesterol are the second amphiphile these compounds contribute very little to the echo at 2τ [4] and consequently also to D. For the soap-decanol systems, on the other hand, it is difficult to judge which amphiphile contributes most to the measured D. This problem could, for example, be solved by using perdeuterated decanol, but in the present work D is regarded as a general amphiphile diffusion constant.

From the values of the diffusion constants presented in table 1 a number of interesting conclusions can be drawn:

Table 2
Diffusion coefficient for some hydrocarbons and alcohols [a]

Compounds	Temp. (°C)	$D \times 10^{10}$ m²/s
n-octane	25	22.5
n-nonane	25	17.0
n-decane	25	13.1
n-octadecane	50	4.6
n-propyl alcohol	25	5.1
n-butyl alcohol	25	4.3
n-octyl alcohol	25	1.4
n-decyl alcohol [b]	25	0.75

[a] From ref. [19].
[b] From ref. [1].

a) A comparison with the D-values for hydrocarbons and alcohols summarized in table 2 reveals that the amphiphile diffusion is considerably slower than for the corresponding alkane but of the same order of magnitude as in alcohols.

b) For the monoglycerides D_L changes by a factor of four when going from a C_8 to a C_{18} carbon chain, which is a chain length dependence rather weaker than that found for alkanes.

c) In the octanoate systems no counter-ion dependence is detected in the amphiphile diffusion.

d) The D_L value found in the lamellar egg lecithin-cholesterol system is the same as was found in a previous ESR spin-label study [6] for a sample with similar composition.

e) In most cases the $D_{\|}$ values found in cubic phases are slightly smaller than the D_L values for the corresponding lamellar systems. This corraborates the assumption that only diffusion in one direction contributes to the transport of spins in these phases. An exception is the $D_{\|}$ value calculated for the C_{18}-monoglyceride. However, for this system the structure of the phase is under debate [15] and it is quite possible that instead eq. (5) should be used to calculate $D_{\|}$.

f) Measurements of D in cubic phases give the possibility of determining if the structure is such that the amphiphile is free to diffuse over macroscopic distances or if the diffusion is restricted in some way [3]. The former would be the case in the rod network structure

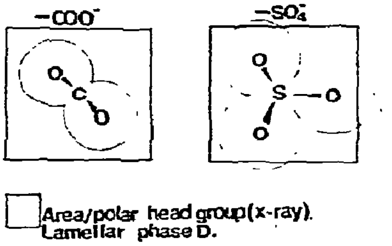

Fig. 4. Schematic illustration of the relation between area per polar head-group and Van der Waals radii in lamellar octanoate and octylsulphate.

while for spherical micellar type aggregates packed in a cubic lattice diffusion between aggregates should be restricted. For all but one of the cubic phases the amphiphile diffusion is unrestricted. However, in the system C_7COONa-octane-D_2O the *D*-value is so small that it is only compatible with a phase structure, where the amphiphile diffusion is restricted. This finding is compatible with the proposed structure of the phase [16]. It is probable that the measured diffusion in this phase is due to the finite concentration of monomers in the water regions. The low value for *D* shows that one can neglect the contribution from the monomers in the other octanoate systems.

g) There are surprisingly large differences between the D_L-values for the C_8-systems studied. The diffusion is a factor of ten faster in C_8NH_3Cl than in C_8SO_4Na with the D_L-values for C_8COOX coming in between. These differences in diffusion rate may be attributed to the difference in packing in the polar head region. The area per polar group *S* as determined in X-ray scattering studies [17,18] shows in fact a similar variation with composition of the lamellar phase. The values of *S* for sulphate and carboxylate are practically the same ($S \approx 25$ Å^2), while the sulphate group is considerably larger as illustrated in fig. 4. There is thus a tighter packing of the polar head groups in the sulphate system and this should also cause higher barriers for diffusional motion. For C_8NH_3Cl $S = 32$ Å^2 giving an even looser packing and a faster diffusion rate.

It has thus been shown that amphiphile translational diffusion coefficients contain information about phase structure and basic molecular interactions. Perhaps most important is, however, the observation that the diffusion in the lamellar systems is as fast as in ordinary liquids of similar composition, a fact that is of the greatest importance for the understanding of transport processes within biological membranes.

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